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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/714,185	11/17/2000	Richard A. Goldsby	50195/009002	5411

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CLARK & ELBING LLP
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EXAMINER	
CROUCH, DEBORAH	
ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 06/15/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/714,185	GOLDSBY ET AL.	
	Examiner	Art Unit	
	Deborah Crouch, Ph.D.	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 April 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-32* is/are pending in the application.

4a) Of the above claim(s) *23-32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-22* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *17 November 2000* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

Applicant's election without traverse of group I, claims 1-22 in the reply filed on April 5, 2004 is acknowledged. Claims 1-32 are pending. Claims 23-32 are withdrawn from consideration.

An Information Disclosure Statement has not been received.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-22 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claims are directed to methods for producing a clone ungulate comprising a disruption of both copies of the IgM gene and transgenic ungulates. Applicant states that technical problems prevented the production of ungulates that contained a disruption of an IgM gene, but that they have solved this problem (specification, page 5, lines 19-19-23). The specification further states that conventional protocols for producing double knockouts in primary cell culture has been uncertain and difficult because primary cells have a limited life span (specification, page 5, lines 23-25). However, the specification never discloses a method or protocol for overcoming these barriers. The specification only teaches the use of fibroblasts to knockout one IgM allele (specification, page 27). Thus, the specification only provides guidance for the use of fibroblasts to knockout expression of a single IgM gene. Further, it was well known in the art at the time of filing, that fibroblasts were the only somatic cell type know to have sufficient divisions in culture to permit selection of the disrupted IgM gene or allele. It was also known in the art at the time of filing that producing

a transgenic knockout animal by disrupting the gene of interest in fibroblasts and then performing nuclear transfer to produce an animal which lacks expression of the gene was unpredictable.

With regards to choice of cell for disruption of a gene of interest, the art taught at the time of filing that the cell would need to undergo significant divisions in the selection process. Clark teaches that only primary somatic cells have been used to successfully in gene targeting experiments to produce livestock having a disrupted gene of choice (Clark, page 265, col.2, parag. 1, lines 12-15.) In addition, Clark teaches that about 45-population doubling are required to generate targeted cells (Clark, page 268, col. 2, parag. 1, lines 1-5). Dennig teaches, unlike ES cells, primary cells have limited proliferation capacity and any genetic modifications and nuclear transfer must be accomplished prior to senescence (Denning, page 222, col. 1, lines 5-8). To further complicate matters, successful nuclear transfer in livestock has only been accomplished using primary somatic cells (Denning, page 224, col. 1, parag. 2, lines 1-4). In a study of sheep and goat primary somatic cells, Denning found that of primary somatic cells, fibroblasts were the only cells that either grew at all from the primary cell source or has sufficient population doublings for the selection required in targeted gene transfer. Sheep primary cell cultures primarily were composed of fibroblasts after the third passage or about 12 doublings (Denning, page 224, col. 2, lines 11-13). Further, a comparison of separate Black Welsh sheep primary cell fibroblast cultures showed vast differences in the number of doublings prior to senescence; 110 doublings versus 40 doublings (Denning, page 224, col. 2, lines 16-19). In a similar analysis of pig primary cultures, fibroblasts, as in the sheep study, become the predominant cell after three passages, but, unlike sheep, pig fibroblasts underwent a crisis after 40 population doublings and had an unstable karyotype (Denning, page 224, col. 2, parag. 4 line 4 to page 225, col. 1, line 8). Additional studies of cell cultures prepare from fetal pig organs (gut, kidney, lung

and mesonephros showed that these cells senesced or entered crisis after even fewer doublings than the fibroblast cultures (page 225, col. 1-2, bridg. sent.). The art further taught at the time of filing, that the even if sufficient population doublings could be achieved for selection, many of the pure sheep targeted clones senesced before they could be expanded for nuclear transfer, meaning that targeting frequency was lower than expected (page 228, col. 1-2, bridg. sent.). Similar experiments in pigs demonstrated that all the clones senesced, and no targeted cells for nuclear transfer were obtained. Denning also points to their own work in producing sheep comprising a disruption of the α1,3-galactosyltransferase gene, live births were achieved but the animals died within two weeks of birth (Denning, page 230, col. 1, parag. 2, lines 1-8). However, Denning reports that McCreath achieved live birth and survival of two gene targeted sheep with disruptions in different genes (Denning, page 230, col. 1, parag. 2, lines 9-12). Based on the analysis of Denning, it is possible that for gene targeted sheep, the success depends on unknown factors, whereas in pigs, the use of fibroblasts to produce gene-targeted pigs is not possible (Denning, page 230, col.1, parag. 1, lines 7-13). In other words, at the time of filing, the skilled artisan would have regarded the production of gene targeted sheep and pigs as being upredicatable requiring an undue amount of experimentation without a predictable degree of success. Further, given that fibroblasts were the only cells shown to divide a sufficient number of times in sheep to provide cells for nuclear transfer, fibroblasts, derived from the mesoderm, would be the only cell useful for the presently claimed invention. The claims are broadly drawn to any cell type. However, the unpredictability shown in Denning and McCreath concerning live birth of gene-targeted sheep would make sheep also unpredictable. Since the art at the time of filing clearly indicated that method for producing two gene targeted livestock animals were unpredictable, it would be reasonable to extend the unpredictability to the genus livestock absent evidence to the contrary. It is noted that

the specification discloses that several fetuses heterozygous for an IgM gene disruption were made (specification, pages 31-34). However, there is no report of a live birth surviving a useful length of time, in this instance surviving sufficiently to be reconstituted with hematopoietic stem cells and produce useful antibody to specific antigens. This is important because, in sheep, the only gene target animal produced by nuclear transfer near the time of filing, exhibited sufficient fetal and postnatal death that Denning achieved no living animals and McCreath achieved two for one gene. The unpredictable nature of gene targeting in somatic cells and combined with nuclear transfer to produce a gene-targeted ungulate is clearly established.

The claims state that the nuclear transfer procedure can use DNA as the donor material. This is not enabled as isolated DNA would contain nicks or breaks and thus be unsuitable for nuclear transfer. Further, isolated DNA has lost its chromosomal structure, and nuclear transfer by its nature requires intact chromosomes rather than a mass of destructured DNA. It was known at the time of filing that nuclear transfer required a diploid chromosomal intact complement.

The art at the time of filing held that the phenotype achieved in the production of transgenic gene targeted mice was unpredictable for the phenotype either expected or desired. Mice with a disruption in the g_c gene were intended to be a model for X-linked severe combined immunodeficiency (XSCID), but display a variety of unexpected traits (Leonard, abstract). These knockout mice were expected to have thymocytes with decreased proliferation in response to stimulation with antibodies, but the thymocytes proliferated normally (page 105, line 7). Additionally, despite a known role for the PLP gene based on spontaneous mutations in the gene, the knockout mouse failed to display any of the expected phenotypes (Griffiths, page 350, last paragraph). Also, Two mutations produced by homologous recombination in two different locations of the N-myc gene

produce two different phenotypes in mouse embryonic stem cells, one leaky and one null (Moens, page 486, column 1, first full paragraph). The examiner realizes that this data is only available for mice, and the examiner realizes that mice are not considered ungulates. However, there is no biological reason why this unpredictability would not carry over to all gene-targeted animals. Thus, absence results to the contrary obtaining an ungulate lacking function IgM is unpredictable because the phenotype desired is not necessarily the phenotype desired. This is shown even when gene function is fully understood.

As disclosed in the specification, the claimed ungulates lack B-cells and antibodies produced by them. The disruption of the IgM gene does not affect T-cell development, nor is the development of macrophages or Nk cells affected. These cells alone can mount a host versus graft response to the heterologous bone marrow, effectively preventing the production of antibodies by the destruction of the bone marrow graft through a lymphocyte and cellular response alone. Nk cells, as well T-cells and macrophages all release TNF, a major cytokine in the destruction of a foreign bone marrow graft. The specification does not provide any guidance on preventing host versus graft disease sufficiently to produce antibodies. (specification, pages 4-5, bridg. parag.)

However, even if applicant establishes that an ungulate lacking functional IgG due to a disruption of the IgG gene can be made, there is unpredictability in the production of antibodies from the ungulate reconstituted with hematopoietic stem cells from humans and other mammals. Cows, sheep and pigs have a relatively small number of functional germline V-genes, which imposes constraints in the generation of antibody diversity as compared with animals such as humans and mice that possess a large pool of divergent VDJ genes that cause significant diversity. In sheep, antibody diversity takes place in the Ileal Payer's patches, where somatic hypermutations take place during B cell development. Bovine B cells develop without the influence of maternal antibodies, and selective forces

operating during B cells development are different from those observed in mice and humans where maternal antibodies influence the developing B cell repertoire. (see Kaushik, pages 347 and 348, col. 1). Thus, it is not clear that an IgM null bovine, reconstituted with hematopoietic stem cells from either mouse or human, as an example, would develop mature B-cells. In humans, B-cells are made in the bone marrow and travel to the lymph nodes for maturation into particular antibody secreting cells. The B-cells reaching the lymph node are committed to a certain antibody lineage. It appears that in bovines, initial B-cell maturation occurs in the Ileal Payer's patch, as that is where somatic hypermutation occurs. (see Parng, pages 5478 and 5479). Since the B-cell maturation process is so different in bovines from that in humans, it is not evidence that human B-cells, for example, leaving the bone marrow, would enter the bovine Ileal Payer's patch or if the cells do enter the lymphoid tissue, that the proper cytokines or antigen presenting cells would be present to cause the production of human antibodies to a particular antigen. Further, human B-cells home to the lymph node for maturation in humans. There is no evidence that human B-cells would home to bovine lymph nodes or if they do, that they will mature into B-cells expressing the desired antibody.

Thus, for the reasons present above, at the time of the instant invention, the skilled artisan would have needed to engage in an undue amount of experimentation without a predictable degree of success to implement the claimed invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 9 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 9 is improperly dependent on claim 8. Claim 8 does not contain subject matter concerning the introduction of hematopoietic stem cells.

The claims are free of the prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Deborah Crouch, Ph.D. whose telephone number is 571-272-0727. The examiner can normally be reached on M-Th, 8:30 AM to 7:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson can be reached on 571-272-0408. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Deborah Crouch

Deborah Crouch, Ph.D.
Primary Examiner
Art Unit 1632

June 12, 2004